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Award Number: W81XWH-04-1-0335

TITLE: Prostate Cancer Models of Genomic Instability

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REPORT DATE: March 2005

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-03-2005	Annual Summary	1 Mar 2004 – 28 Feb 2005

4. TITLE AND SUBTITLE
Prostate Cancer Models of Genomic Instability

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-04-1-0335

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Fred Hutchinson Cancer Research Center
Seattle, WA 98109-1024

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT:

No Abstract Provided

15. SUBJECT TERMS
No Subject Terms Provided

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	8	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

Genomic instability is a common feature of human cancer, and is associated with aberrant mitosis, aneuploidy, and other chromosomal irregularities [1]. Genomic instability likely arises from centrosome abnormalities that induce spindle defects, leading to imbalanced chromosomal segregation. In fact, centrosome anomalies arise in early prostate cancer and occur more frequently in high-grade cancers [2]. While the molecular events of genomic instability are not fully understood, their characteristics are tightly associated with aberrant expression of proteins controlling DNA damage response and cell cycle checkpoints such as the mitotic kinase Aurora-A and -B, and checkpoint protein Bub1. Overexpression of Aurora-A and -B are overexpressed in many transformed and cancer cell lines and their levels increases as a function of tumor state in many different types of cancers [3, 4]. Overexpression of Aurora-A kinase induces centrosome amplification, aneuploidy, abnormal cell cycles, and transformation of p53-deficient mammalian cells [5]. Inactivational mutation in Bub1, although rare, functions dominantly in some cancers and induces chromosomal instability and increase aneuploidy [6, 7]. In addition, recently studies have shown that inactivation of Bub1 activity is essential for SV40 large T-antigen-induced neuplastic transformation [8].

Key Research Accomplishments

- Shown that the steady-state expression levels of Aurora-A and -B, and Bub1 in human prostate cancer cell lines are positively correlates with their tumorigenecity and invasive potential.
- Shown that TRAMP cell lines express high levels of Aurora-A and -B, and low levels of Bub1.
- Shown that TRAMP mice express high level of Aurora-A and Aurora-B in the prostatic tumors while only low to moderate level (AP=DP<LP<VP) of the proteins were found in the prostatic tissue of age-matched non-transgenic mice.
- Identified a novel mis-sense mutation of the p53 gene (ΔT812) in TRAMP cell lines that may play a significant role in tumorigenesis of the prostate.

Reportable Outcomes

I) Status of Aurora-A, Aurora-B and Bub1 in Human and TRAMP tumors

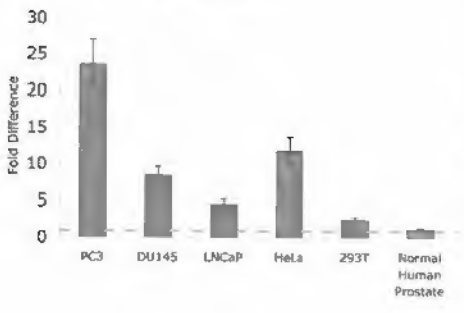
We have demonstrated that the steady-state expression levels of Aurora-A, Aurora-B, and Bub1 in LNCaP, DU145 and PC3 human prostate cancer cell lines is positively correlated with their tumorigenecity and invasive potential (Figure 1). We have also demonstrated that TRAMP cell lines express high levels of Aurora-A and Aurora-B and low levels of Bub1 (Figure 2). In addition, TRAMP mice express high level of Aurora-A and Aurora-B in the prostatic tumors while only low to moderate level (AP=DP<LP<VP) of the proteins were found in the prostatic tissues of age-matched non-transgenic mice (Figure 3 and 4).

Figure 1

A

	Doubling time (h)	Tumorgenecity	Fold Difference					
			Aurora A	(95% C.I.)	Aurora B	(95% C.I.)	Bub1	(95% C.I.)
PC3	50.0	+++	23.43	20.40-26.92	48.35	44.28-52.79	37.67	33.36-42.54
DU145	36.0	++	8.32	7.26-9.52	32.98	30.42-35.75	13.56	12.01-15.30
LNCaP	34.0	+	4.46	3.87-5.15	21.09	19.21-23.15	10.88	9.56-12.38
HeLa	37.0	-	11.60	9.86-13.64	47.29	42.09-53.13	7.21	6.23-8.35
293T	20.0	-	2.31	1.99-2.68	54.60	48.64-61.28	3.70	3.24-4.23
Normal Human Prostate	NA	-	1.00	0.83-1.20	1.00	0.91-1.10	1.00	0.85-1.17

B



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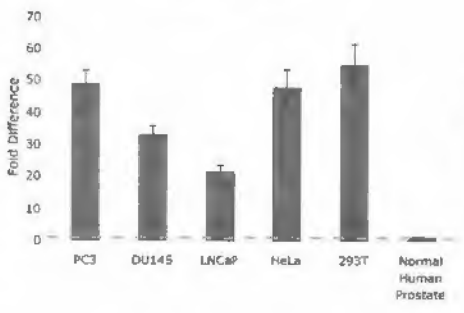

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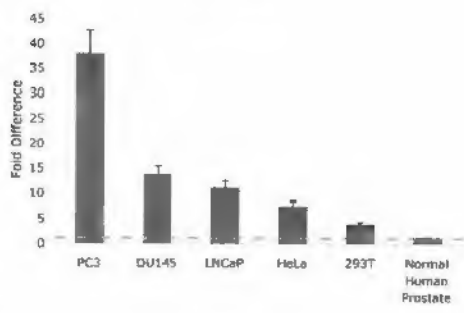
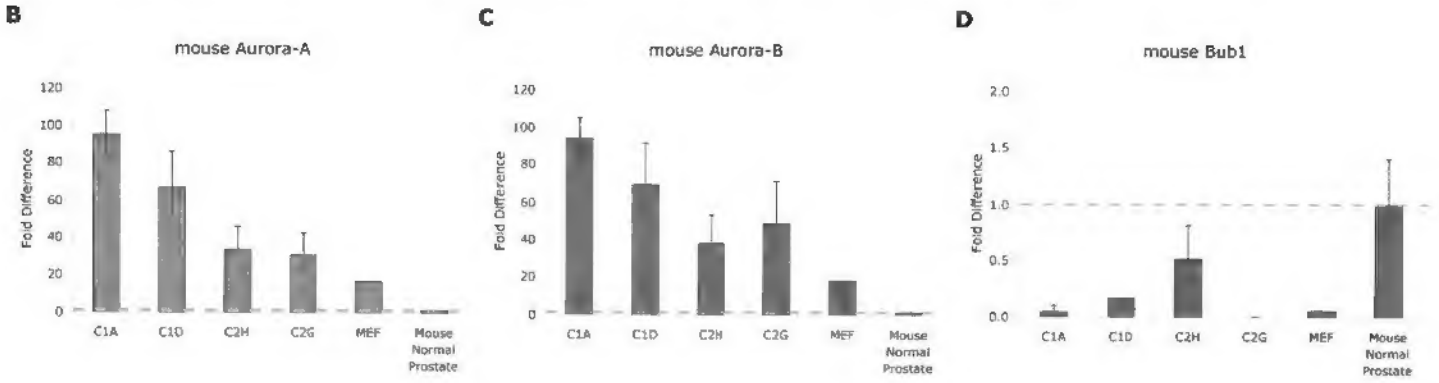


Figure 2

A

	Doubling time (h)	Tumorgenecity	Fold Difference					
			Aurora A	(95% C.I.)	Aurora B	(95% C.I.)	Bub1	(95% C.I.)
C1A	16.7	- (0/12)	94.79	(83.46-107.65)	93.70	(83.67-104.93)	0.04	(0.01-0.10)
C1D	15.1	- (3/7)	66.26	(51.31-85.56)	68.59	(51.70-90.99)	0.15	-
C2H	12.6	+ (4/5)	33.32	(24.21-45.86)	37.62	(26.71-52.98)	0.51	(0.32-0.81)
C2G	10.2	+ (5/5)	30.38	(21.88-42.18)	48.50	(33.22-70.80)	ND	-
MEF	17.0	NA	16.00	-	17.75	-	0.05	-
Mouse Normal Prostate	NA	NA	1.00	(0.83-1.20)	1.00	(0.83-1.21)	1.00	(0..72-1.39)



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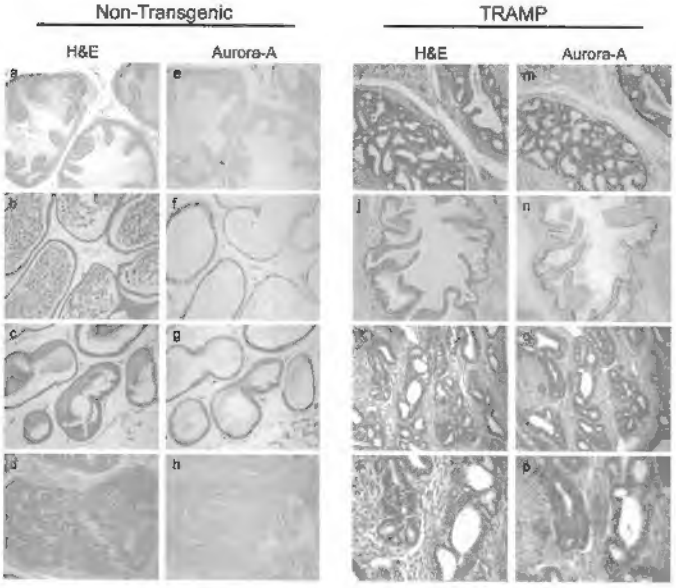
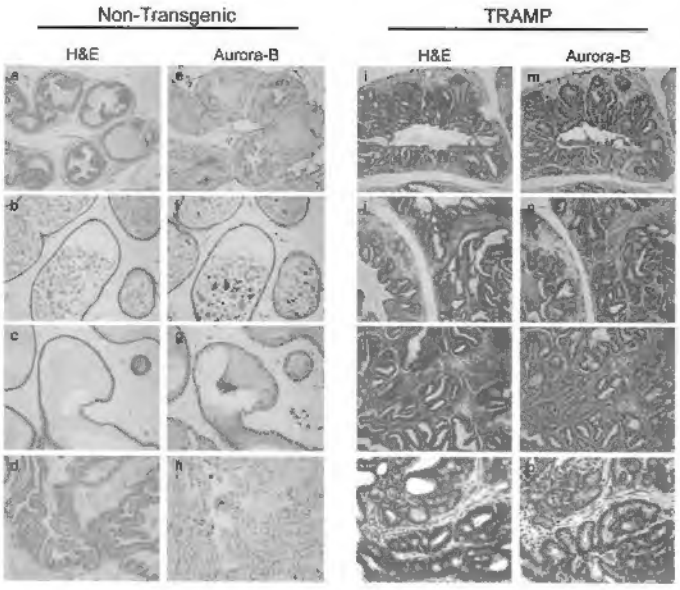


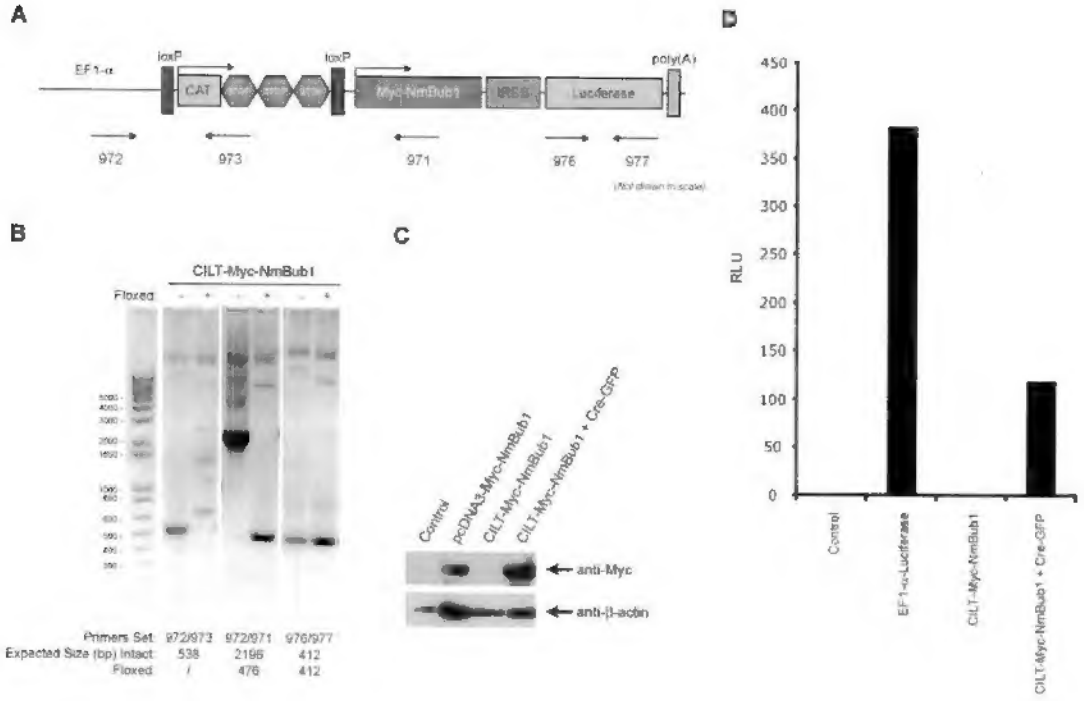
Figure 4



II) Construction of Cre-Inducible Luciferase-linked Transgenic Mice for NmBub1 and Aurora-B

We have generated a construct of pEF1α-loxP-CAT-loxP-MCS-IRES-Luciferase (designated *Cre*-Inducible Luciferase-linked Transgenic (CILT) system) that was designed to induce expression of both the transgene and luciferase enzyme by *Cre*-mediated recombination. We then subcloned mouse Myc-tagged dominant-negative Bub1 (Myc-NmBub1) and V5-tagged rat Aurora-B (V5-rAuroraB) into the CILT system and generated two constructs: CILT-Myc-NmBub1 and CILT-V5-AuroraB. These constructs have been sequence-verified (data not shown). Functional analyses have confirmed their effectiveness to express both the transgene and luciferase enzyme after *Cre*-induced floxing of the construct (Figure 5 and 6). These two constructs have been isolated, purified, microinjected into C57BL6/CBA ES cells and transplanted into pseudo-pregnant C57BL6/CBA females. Four individual CILT-Myc-NmBub1 founder lines (designated NMG1859, NMG1865, NMG1869 and NMG1870) and three individual CILT-V5-rAuroraB founder lines (designated NMG1997, NMG2000 and NMG2003) were identified. The transgene copy numbers of these founder lines as determined by qPCR are shown in Table 1. Characterizations of the transmittance and pathobiology of these founder lines are currently underway.

Figure 5



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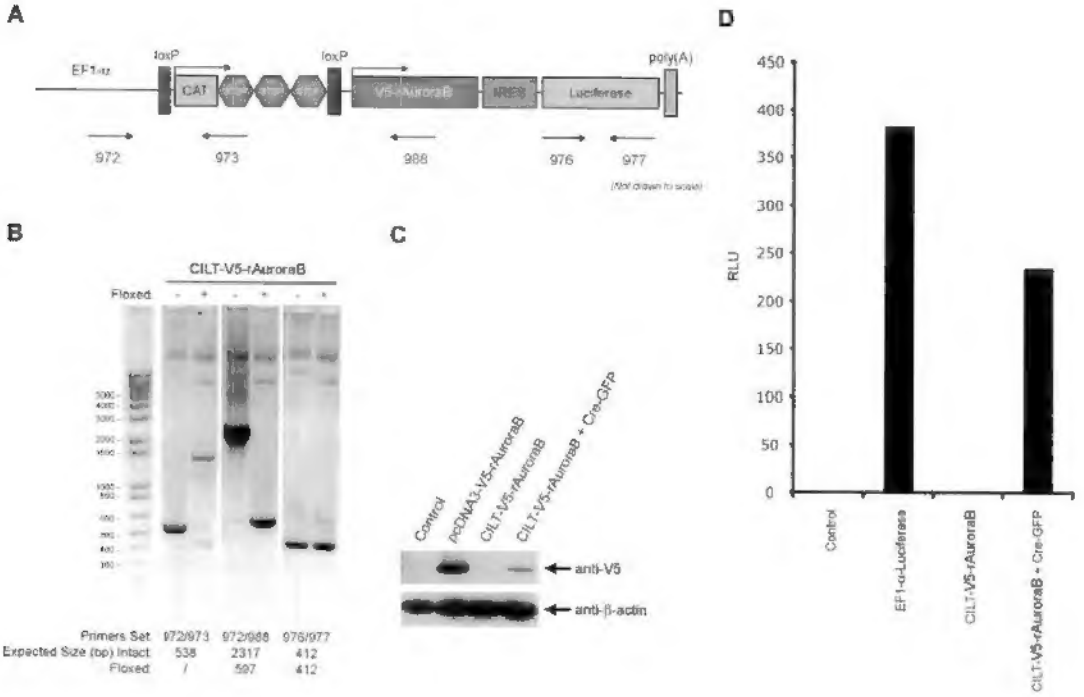


Table 1

Construct	Founder Line	Transgene Copy Number (per diploid)	Transmittance
CILT-Myc-NmBub1	NMG1859	1.14 ± 0.09	To be determined
	NMG1865	2.15 ± 0.30	To be determined
	NMG1869	3.16 ± 0.10	To be determined
	NMG1870	3.19 ± 0.96	To be determined
CILT-V5-rAuroraB	NMG1997	13.98 ± 1.02	To be determined
	NMG2000	4.13 ± 1.79	To be determined
	NMG2003	0.64 ± 0.09	To be determined

III) Status of p53 in TRAMP tumors

We have amplified, isolated and sequenced p53 cDNA from different TRAMP cell lines and identified a point deletion at nucleotide T812 that leads to a frameshift mutation at codon 272 and a premature stop at codon 341 where part of the DNA binding domain (codon 102-292) and all of the tetramerization (codon 323-356) and negative regulation (codon 363-391) domains were aberrated (Figure 7).

Figure 7

A. Mouse p53(wt)

MTAMEESQSDISLELPLSQETFSGLWKLLPPEDILPSPHCMDDLLLPQDVEEFFEGPSEALRVSGAPAAQDPVTETPGPVAPAPATPW
PLSSFVPSQKTYQGNYGFHLGFLQSGTAKSVMCTYSPPLNKLFCQLAKTCPVQLWVSATPPAGSRVRAMAIYKKSQHMTEVVRRCP
HHERCSDGDGLAPPQHLIRVEGNLYPEYLEDRQTFRHSVVVPYEPPEAGSEYTTIHYKYMCNSSCMGGMNRRPILTIITLEDSSGNLL
GRDSFEVRV[271] ***CACPGRDRRTEEENFRKKEVLCPELPPGSAKRALPTCTSASPPQKKKPLDGEYFTLKIRGRKRFEMFRELNEALE***
LKDAHATEESGDSRAHSSYLKTKKGQSTSRHKKTMVKKVGPDSD*[391]

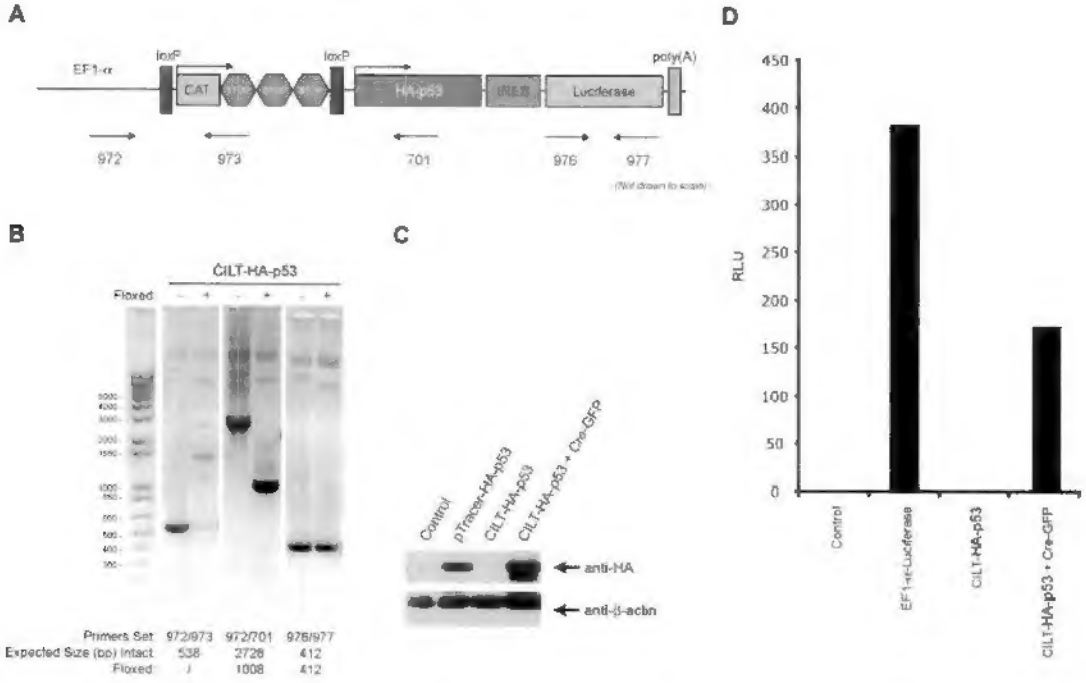
B. Mouse p53(ΔT812)

MTAMEESQSDISLELPLSQETFSGLWKLLPPEDILPSPHCMDDLLLPQDVEEFFEGPSEALRVSGAPAAQDPVTETPGPVAPAPATPW
PLSSFVPSQKTYQGNYGFHLGFLQSGTAKSVMCTYSPPLNKLFCQLAKTCPVQLWVSATPPAGSRVRAMAIYKKSQHMTEVVRRCP
HHERCSDGDGLAPPQHLIRVEGNLYPEYLEDRQTFRHSVVVPYEPPEAGSEYTTIHYKYMCNSSCMGGMNRRPILTIITLEDSSGNLL
GRDSFEVRV[271] ***VPALGETAVQKKKISAKRKSFALNCPQGAQRERCPPAQAPLPRKRKNHLMESISPSRSAGVNASRCSGS****[341]

IV) Construction of Cre-Inducible Luciferase-linked Transgenic Mice for p53 mutants

We have subcloned HA-tagged mouse p53 (wildtype) into the CILT system and generated the CILT-HA-mp53(wt) construct. Functional analyses have confirmed its effectiveness to express both the wildtype p53 protein and luciferase enzyme after *Cre*-induced floxing of the construct (Figure 8). CILT-constructs with different HA-taggged p53 mutants (R172H, R172L, G242S, R279Q) were then generated by site-directed mutagenesis. These constructs have been sequence-verified and their effectiveness to express both the p53 protein and luciferase enzyme has been confirmed (data not shown). These constructs will be isolated, purified, microinjected into C57BL6/CBA ES cells and transplanted into pseudo-pregnant C57BL6/CBA females for the identification and characterization of their founders.

Figure 8

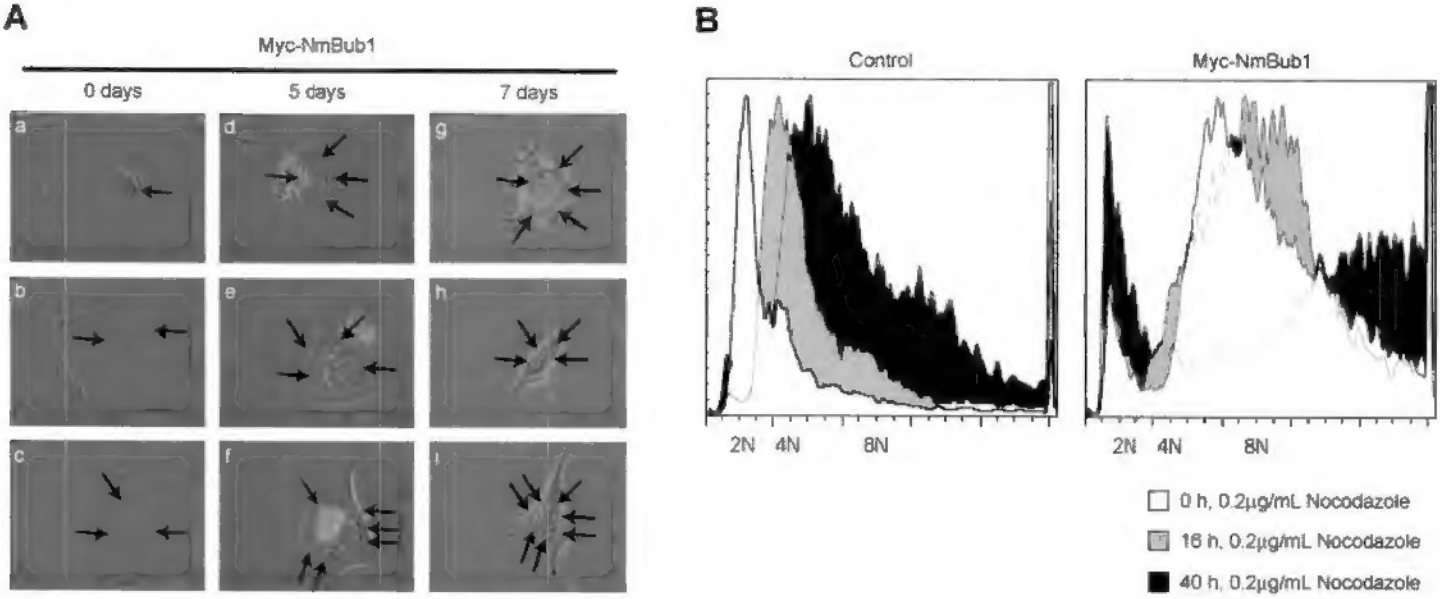


V) Effects of Transgene on Aneuploidy in TRAMP C1A Mouse Prostate Cancer Cells

We have transfected TRAMP C1A mouse prostate cancer cells with the pcDNA3.1-Myc-NmBub1 construct. Upon expression of the Myc-tagged NmBub1, C1A cells have shown a significant increase in aneuploidy (Figure 9A). This increase was further exemplified after treatment with the microtubules destabilizing agent

Nocodazole (0.2μg/mL) (Figure 9B). The possible synergistic effects of NmBub1, Aurora-B and different p53 mutants on prostate cancer cell's aneuploidy are currently under investigation.

Figure 9



Summary

Construct	Functional Analyses	Founder Lines Established?	Mouse Pathobiology	Effects on aneuploidy in TRAMP cells
CILT-Myc-NmBub1	Completed	Y	In Progress	Y
CILT-V5-rAurora-B	Completed	Y	In Progress	In Progress
CILT-HA-mp53(wt)	Completed	N	-	In Progress
CILT-HA-mp53(R172H)	Completed	N	-	In Progress
CILT-HA-mp53(R172L)	Completed	N	-	In Progress
CILT-HA-mp53(G242S)	Completed	N	-	In Progress
CILT-HA-mp53(R279Q)	Completed	N	-	In Progress

Conclusions

Data supports our hypothesis that Aurora-A, Aurora-B and Bub1 play important roles in the initiation and progression of prostate cancer. Transgenic models are currently being established and characterized.

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Publications

Lee EC, Ruiz M., Greenberg N.M. Prostate Cancer and Genomic Instability. [Abstract of poster presented at the 11th Prostate Cancer Foundation Scientific Retreat, Lake Tahoe, Nevada, USA. October 2004]